Phytotaxa 205 (1): 001–020
www.mapress.com/phytotaxa/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.205.1.1

Taxonomic evaluation of *Miscanthus nudipes* (Poaceae) based on morphological and molecular evidence

HONG-ZHENG MA[1, 2], ZHE CAI[2], FU-MIN ZHANG[2], HUI ZHANG[2], SONG GE[2], SI-LAN DAI[1*] & WEN-LI CHEN[2*]

[1]*College of Landscape Architecture, Beijing Forestry University, Beijing 100083, P. R. China*
[2]*State Key Laboratory of Systematic and Evolutionary Botany, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, P. R. China*
** Authors for correspondence: WEN-LI CHEN, chenwl@ibcas.ac.cn; SI-LAN DAI, silandai@sina.com*

Abstract

Miscanthus species including the famous energy plant *M.* × *giganteus* are considered to be second-generation energy crops. *Miscanthus nudipes* is an endemic species from Himalaya-Hengduan Mountains and provides important germplasm for *Miscanthus* breeding given its high tolerance to frost and drought. However, the taxonomy of this species remains controversial in terms of intraspecific classification, with one to nine subspecies or species recognized previously. The variation patterns of the morphological characters are not understood clearly as there have been no intensive analyses of the morphological variation across individuals and populations. Here, by sampling 15 natural populations from the entire distribution area of *M. nudipes*, we investigate patterns of population variation based on 39 quantitative and nine qualitative morphological characters and 14 microsatellite loci, with the aim to provide a reliable taxonomic treatment of this important species. Morphological analyses from ANOVA, UPGMA and PCA indicate that *M. nudipes* populations show significant differences between the Himalayas and Hengduan Mountains. A neighbor-joining tree and principle coordinates analysis of the microsatellite data support the results of the morphological analyses. Based on our results, we recognize two subspecies under *Miscanthus nudipes* (*M. nudipes* subsp. *nudipes* and *M. nudipes* subsp. *yunnanensis*) and provide a taxonomic treatment for the species. We propose three diagnostic characters for the subspecies identification, i.e., the ratio of callus hair length to spikelet length, the hairiness of the panicle axis and the hairiness of the peduncle.

Keywords: classification, Hengduan Mountains, Himalayas, morphology, microsatellite markers

Introduction

Lignocellulosic-based ethanol is referred to as second-generation biofuel. It is a better choice than starch and sugar-based ethanol in temperate regions, and may play an essential role in reaching the target to replace petroleum-based fuels in the future (Yuan *et al.* 2008). The genus *Miscanthus* Andersson (1855: 165) is known for having potential in developing second-generation energy crops in marginal and degraded land (Beale & Long 1995, Clifton-Brown *et al.* 2008, Yan *et al.* 2012). Some *Miscanthus* species have been cultivated and show excellent potential to offset gasoline and CO_2 emissions from petroleum (Heaton *et al.* 2008). However, their low frost and drought tolerances results in low survival rate in degraded land (Jørgensen & Schwarz 2000, Yan *et al.* 2012), which substantially restrict the cultivated area of this crop and reduces its biomass productivity (Yan *et al.* 2012).

Miscanthus consists of more than 10 species distributed across the Old World continents and harbors a high level of genetic variation (Clifton-Brown *et al.* 2008, Yan *et al.* 2012, Yook *et al.* 2014). The morphologically similar species *M. nudipes* (Grisebach 1868: 92) Hackel (1889: 109) and *M. nepalensis* (Trinius 1833: 333) Hackel (1889: 104) are distinguished from other *Miscanthus* taxa by stamen number (two vs. three; Chen & Renvoize 2006, Sun *et al.* 2010) and chromosome number (n=20 vs. 2n=38; Mehra & Sharma 1975), and are treated as *Miscanthus* sect. *Diandra* Keng (1959: 748) or *Miscanthus* subgen. *Diandranthus* (Liou 1987: 308) Sun & Lin (2010: 206). Liu (1997) argued that stamen number is a very important character and separated these two–anther species from *Miscanthus* as a new genus, *Diandranthus* Liou (1987: 308). *Miscanthus nudipes* and *M. nepalensis* are the only *Miscanthus* members endemic to

Accepted by Jeff Saarela: 19 Mar. 2015; published: 10 Apr. 2015

szechuanensis Keng (1959: 754) *nom. nud.*; in Zhong (1982: 80) *nom. valid.* ≡ *Diandranthus szechuanensis* (Keng ex Zhong) Liou (1994: 2297) *comb. invalid.* Type:—CHINA. Sichuan, Leibo Xian: 1500m. *T.T. Yü, 3507* (holotype PE!). =*Diandranthus aristatus* Liu (1997: 18) *nom. nud.* Type:—not designated but the locality is cited as 'Sichuan, Daofu' in Liu (1997: 18).

Amended morphological description:—Peduncle and panicle axis glabrous. Panicle 6.9–20.4 cm long, 1.0–15.1 cm wide; axis 1.7–17.1 cm long. Spikelets 3–6.9 mm long, 0.4–1 mm wide, awned; callus hairs 0.8–5 mm long, white. The ratio of callus hair length to spikelet length less than 0.65.

Distribution:—Guizhou (1000–1500m), Sichuan (1400–3400m) and Yunnan (1750–3300m) in China.

Acknowledgements

We thank Si-si Huang and Xiao-xia Gao from College of Landscape Architecture in Beijing Forestry University for their contribution in morphological characters measurement. Special thanks are due to Prof. Xiang-yun Zhu for helpful discussion. This study was supported by the Chinese Academy of Sciences (KZCX2-YW-034) and the National Natural Science Foundation of China (31470300, 91131902, 31070168).

References

Adati, S. & Shiotani, I. (1962) The cytotaxonomy of the genus *Miscanthus* and its phylogenic status. *Bulletin of the Faculty of Agriculture* 25: 1–14.

Amalraj, V.A. & Balasundaram, N. (2006) On the taxonomy of the members of '*Saccharum complex*'. *Genetic Resources and Crop Evolution* 53: 35–41.
http://dx.doi.org/10.1007/s10722-004-0581-1

Andersson, N.J. (1855) Om de med *Saccharum*beslägtade genera. *Öfversigtaf Förhandlingar: Kongliga. Svenska Vetenskaps-Akademien* 12: 151–168.

Beale, C.V. & Long, S.P. (1995) Can perennial C_4 grasses attain high efficiencies of radiant energy conversion in cool climates? *Plant, Cell and Environment* 18: 641–650.
http://dx.doi.org/10.1111/j.1365-3040.1995.tb00565.x

Barrowclough, G.F. (1982) Geographic variation, predictiveness, and subspecies. *Auk* 99: 601–603.

Bhattacharyya, A. & Chaudhary, V. (2003) Late-summer temperature reconstruction of the Eastern Himalayan Region based on tree-ring data of *Abies densa*. *Arctic, Antarctic and Alpine Research* 35: 196–202.

Bor, N.L. (1953) Notes on Asiatic grasses: XII, new species. *Kew Bulletin* 1953: 269–276.

Camus, A. (1919) Espèces et variétés nouvelles de Graminées de l'Asie orientale. *Bulletin du Museum National d'Histoire Naturelle* 25: 669–672.

Clark, L.V., Brummer, J.E., Głowacka K., Hall, M.C., Heo, K., Peng, J., Yamada, T., Yoo, J.H., Yu, C.Y., Zhao, H., Long, S.P. & Sacks, E.J. (2014) A footprint of past climate change on the diversity and population structure of *Miscanthus sinensis*. *Annals of Botany* 114: 97–107.
http://dx.doi.org/10.1093/aob/mcu084

Chen, S.L. & Renvoize, S.A. (2006) *Miscanthus* Andersson. *In:* Wu, Z.Y. & Raven, P. H. (Eds.) *Flora of China*. Science Press, Beijing; Missouri Botanical Garden Press, St. Louis, Missouri, pp. 581–583.

Clifton-Brown, J., Chiang, Y.C. & Hodkinson, T.R. (2008) *Miscanthus*: genetic resources and breeding potential to enhance bioenergy production. *In:* Vermerris, W. (Ed.) *Genetic Improvement of Bioenergy Crops.* Springer, New York, pp. 273–294.
http://dx.doi.org/10.1007/978-0-387-70805-8

Defraeye, T., Derome, D., Verboven, P., Jan Carmeliet, J. & Nicolai, B. (2014) Cross-scale modelling of transpiration from stomata via the leaf boundary layer. *Annals of Botany* 114 (4): 711–723.
http://dx.doi.org/10.1093/aob/mct313

Doyle, J.J. & Doyle, J.L. (1987) A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin* 19: 11–15.

Du, F.K., Peng, X.L., Liu, J.Q., Lascoux, M., Hu, F.S. & Petit, R.J. (2011) Direction and extent of organelle DNA introgression between two spruce species in the Qinghai-Tibetan Plateau. *New Phytologist* 192: 1024–1033.
http://dx.doi.org/10.1111/j.1469-8137.2011.03853.x

Excoffier, L., Laval, G. & Schneider, S. (2005) Arlequin ver 3.1: An integrated software package for population genetics data analysis. *Evolution Bioinformatics* 1: 47–50.

Ge, S. & Hong, D.Y. (2010) Biosystematic studies on *Adenophora potaninii* Korsh. complex (Campanulaceae) V. A taxonomic treatment. *Journal of Systematics and Evolution* 48: 445–454.
http://dx.doi.org/10.1111/j.1759-6831.2010.00104.x

Grisebach, A.H.R. (1868) Über die Gramineen hochasiens. *Nachrichten von der Georg-Augusts-Universitaï und der Gesellschaft der Wissenschaften* 1868: 61–93.

Hackel, E. (1889) Andropogoneae. *Monographiae Phanerogamarum* 6: 74–691.

Handel-Mazzetti, H. (1936) *Anthophyta*. Verlag von Julius Springer, Vienna, pp. 1306–1307.

Hanley, M.E., Lamont, B.B., Fairbanks, M.M. & Rafferty, C.M. (2007) Plant structural traits and their role in anti-herbivore defence. *Perspectives in Plant Ecology, Evolution and Systematics* 8: 157–178.
http://dx.doi.org/10.1016/j.ppees.2007.01.001

Hardion, L., Verlaque, R., Baumel, A., Juin, M. & Vila, B. (2012) Revised systematics of Mediterranean *Arundo* (Poaceae) based on AFLP fingerprints and morphology. *Taxon* 61: 1217–1226.

Heaton, E.A., Dohleman, F. G. & Long, S.P. (2008) Meeting US biofuel goals with less land: the potential of *Miscanthus*. *Global Change Biology* 14: 2000–2014.
http://dx.doi.org/10.1111/j.1365-2486.2008.01662.x

Hodkinson T.R., Renvoize, S.A. & Chase, M.W. (1997) Systematics of *Miscanthus*. *Aspects of Biology* 49: 189–197.

Ibaragi, Y. (2003) The taxonomy of *Diandranthus* (Poaceae). *Acta Phytotaxonomica et Geobotanica* 54: 109–125.

Irwin, D.E., Irwin, J.H. & Smith, T.B. (2011) Genetic variation and seasonal migratory connectivity in Wilson's warblers (*Wilsonia pusilla*): species-level differences in nuclear DNA between western and eastern populations. *Molecular Ecology* 20: 3102–3115.
http://dx.doi.org/10.1111/j.1365-294X.2011.05159.x

Jørgensen, U. & Schwarz, K.U. (2000) Why do basic research? A lesson from commercial exploitation of *Miscanthus*. *New Phytologist* 148: 190–193.
http://dx.doi.org/10.1046/j.1469-8137.2000.00768.x

Keng, Y.L. (1939) The gross morphology of Andropogoneae (from the standpoint of taxonomy). *Sinenesia* 10: 273–343.

Keng, Y.L. (1959) *Flora Illustralis Plantarum Primarum Sinicarum-Gramineae.* Science Press, Beijing, pp. 748–755.

Lee, Y.N. (1971) Notes on type specimens of *Miscanthus* in Kew Herbarium. *Korean Journal of Plant Taxonomy* 3: 17–18.

Levin, D.A. (1973) The role of trichomes in plant defense. *Quarterly Review of Biology* 48: 3–15.

Li, Z.S., Zhang, Q.B. & Ma, K. (2012) Tree-ring reconstruction of summer temperature for A.D. 1475–2003 in the central Hengduan Mountains,Northwestern Yunnan, China. *Climate Change*110: 455–467.
http://dx.doi.org/10.1007/s10584-011-0111-z

Linnaeus, C. (1753) *Species Plantarum* 1. Imprensis Laurentii Salvii, Holmiae, pp. 54.

Liou, L. (1987) *Diandranthus*. *In*: Wu, C.Y. (Ed.) *Flora Xizangica*. Science Press, Beijing, pp. 308–315.

Liu, K. & Muse, S.V. (2005) Powermarker: An integrated analysis environment for genetic marker analysis. *Bioinformatics* 21: 2128–2129.
http://dx.doi.org/10.1093/bioinformatics/bti282

Liu, L. (1997) *Diandranthus*. *In*: Chen, S.L. (Ed.) *Flora reipublicae popularis sinicae*. Science Press, Beijing, pp. 4–26.

Ma, H.Z., Li, S.S., Ge, S., Dai, S.L. & Chen, W.L. (2011) Isolation of SSR markers for two related second-generation energy crop species, *Miscanthus nepalensis* and *M. nudipes* (Poaceae). *Biodiversity Science* 19: 535–542.
http://dx.doi.org/10.3724/SP.J.1003.2011.08109

Manetas, Y. (2003) The importance of being hairy: the adverse effects of hair removal on stem photosynthesis of *Verbascum speciosum* are due to solar UV-B radiation. *New Phytologist* 158: 503–508.
http://dx.doi.org/10.1046/j.1469-8137.2003.00768.x

McCloud, E.S. & Berenbaum, M.R. (2000) Effects of spring and summer levels of UV-B radiation on the growth and reproduction of a temperate perennial forb. *Plant Ecology* 46: 61–66.
http://dx.doi.org/10.1023/A:1009827222588

Mehra P.N. & Sharma M.L. (1975) Cytological studies in some central and eastern Himalayan grasses: 1 the Andropogoneae. *Cytologia* 40: 61–74.

Michaux, A. (1803) *Flora Boreali-Americana.* Missouri Botanical Garden, London, pp. 54–55.

Moench, C. (1794) *Methodus Plantas horti botanici et agri Marburgensis : a staminum situ describendi*. Officina nova libraria academiae, Marburgi Cattorum, 207 pp.
http://dx.doi.org/10.5962/bhl.title.304

Nei, M. (1987) *Molecular evolutionary genetics*. Columbia University Press, New York, pp. 21–215.

Norusis, M.J. (2007) *SPSS 15.0 guide to data analysis*. Prentice Hall, Upper Saddle River, New Jersey, 610 pp.

Peakall, R. & Smouse, P.E. (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28: 2537–2539.
http://dx.doi.org/10.1093/bioinformatics/bts460

R Development Core Team (2010) *R: A language and environment for statistical computing*. R foundation for statistical computing, Vienna. Available from: http://www. R-porject. org.

Russell, A., Samuel, R., Bogarín, D., Fernando, S., Wijesundera, S., Klejna, V. & Chase, M.W. (2011) Genetic variation and phylogenetic relationships of a pantropical species group in *Polystachya* (Orchidaceae). *Botanical Journal of the Linnean Society* 165: 235–250.
http://dx.doi.org/10.1111/j.1095-8339.2010.01108.x

Sánchez, R., Sepúlveda, R.D., Brante, A. & Cárdenas, L. (2011) Spatial pattern of genetic and morphological diversity in the direct developer *Acanthina monodon* (Gastropoda: Mollusca). *Marine Ecology Progress Series* 434: 121–131.
http://dx.doi.org/10.3354/meps09184

Sun, Q., Lin, Q., Yi, Z.L., Yang, Z.R. & Zhou, F.S. (2010) Taxonomic revision of *Miscanthus s.l.* (Poaceae) from China. *Botanical Journal of the Linnean Society* 164: 178–220.
http://dx.doi.org/10.1111/j.1095-8339.2010.01082.x

Trinius, C.B. (1833) Andropogineorum genera speciesque complures definitionbus novis. *Mémoires de l'AcadémieImperiale des Sciences de St.-Pétersbourg*. *Sixième Série. Sciences Mathématiques, Physiques et Naturelles* 2 (3): 31–337.

Tsukaya, H. & Tsuge, T. (2001) Morphological adaptation of inflorescences in plants that develop at low temperatures in early spring: The convergent evolution of "Downy Plants". *Plant Biology* 3: 536–543.
http://dx.doi.org/10.1055/s-2001-17727

Tobias, J.A., Seddon, N., Spottiswoode, C.N., Pilgrim, J.D., Fishpool, L.D.C. & Collar, N.J. (2010) Quantitative criteria for species delimitation. *Ibis* 152: 724–746.
http://dx.doi.org/10.1111/j.1474-919X.2010.01051.x

Wiegand, K.M. (1910) The relation of hairy and cutinized coverings to transpiration. *Botanical Gazette* 49: 430–444.

Xu, T., Abbott, R.J., Milne, R.I., Mao, K., Du, F.K., Wu, G., Ciren, Z., Miehe, G. & Liu, J. (2010) Phylogeography and allopatric divergence of cypress species (*Cupressus* L.) in the Qinghai-Tibetan Plateau and adjacent regions. *BMC Evolutionary Biology* 10: 194.
http://dx.doi.org/10.1186/1471-2148-10-194

Yan, J., Chen, W.L., Luo, F., Ma, H.Z., Meng, A.P., Li, X.W., Zhu, M., Li, S.S., Zhou, H.F., Zhu, W.X., Han, B., Ge, S., Li, J.Q. & Sang, T. (2012) Variability and adaptability of *Miscanthus* species evaluated for energy crop domestication. *Global Change Biology Bioenergy* 4: 49–60.
http://dx.doi.org/10.1111/j.1757-1707.2011.01108.x

Yook, M.J., Lim, S.H., Song, J.S., Kim, J.W., Zhang, C.J., Lee, E.J., Ibaragi, Y., Lee, G.J., Nah, G. & Kim, D.S. (2014) Assessment of genetic diversity of Korean *Miscanthus* using morphological traits and SSR markers. *Biomass and Bioenergy* 66: 81–92.
http://dx.doi.org/10.1016/j.biombioe.2014.01.025

Yuan, J.S., Tiller, K.H., Al-Ahmad, H., Stewart, N.R. & Stewart, C.N. Jr. (2008) Plants to power: bioenergy to fuel the future. *Trends in Plant Science*13: 421–429.
http://dx.doi.org/10.1016/j.tplants.2008.06.001

Zhao, H., Wang B., He, J., Yang, J., Pan, L., Sun, D. & Peng, J. (2013) Genetic diversity and population structure of *Miscanthus sinensis* germplasm in China. *PLOS ONE* 8: 1–9.
http://dx.doi.org/10.1371/journal.pone.0075672.t001.

Zhong, S.L. (1982) Five new grass species in Sichuan. *Journal of Southwest Agricultural College* 1:75–85.